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Hair cortisol concentrations, as a measure of chronic activity within the hypothalamic-pituitary-adrenal axis, is elevated in dogs farmed for meat, relative to pet dogs, in South Korea.

Running title: Meat farm dogs are chronically stressed

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Abstract:

Human consumption of dog meat continues in some countries as a result of tradition, ritual and claimed medical benefits. In South Korea, it is estimated that over 2 500 000 dogs are eaten annually; however, dog farming is unregulated, as dogs are not classified as livestock, leading to animal welfare concerns. A key component of the physiological stress response is activation of the hypothalamic-pituitary-adrenal (HPA) axis. Cortisol released as a consequence of HPA axis activation is stably deposited in growing biological media, such as hair. Extraction and quantification of hair cortisol can provide a historical record of physiological stress experienced as the hair was growing. By comparison of hair cortisol concentrations in samples

collected from dogs surrendered from meat farms with pet dogs, this study demonstrates that hair cortisol concentrations from dogs rescued from meat farms are over twice as high as pet dogs living in and close to Seoul. This difference was independent of sex, breed and coat colour. Within the farmed dogs there were no significant effects of farm identity, number of dogs/farm or dogs/cage. Within the Korean jindos surrendered from meat farms, hair cortisol was significantly higher in white-coated compared to black-coated dogs but there were no significant differences within or between dogs of other coat colour variants (agouti, brown). These data provide quantitative evidence that dogs in meat farms are kept in conditions associated with poor welfare and identify the need for better welfare laws to protect farmed dogs.

Keywords: animal welfare, dog, dog meat farms, hair cortisol, stress. South Korea

Introduction

The farming of dogs (*Canis familiaris*) for meat production is controversial due to cultural differences and to the role of dogs as companion animals in many cultures. Where dog farming exists, it is often unregulated, with animals maintained in intensive, unsanitary conditions with poor veterinary care throughout and at the end of life (Podberscek 2007; 2009; Kim 2008). In Korea, while dog meat has been eaten for over 2000 years its popularity has varied with dynastic rule and associated changes in religious belief (Podberscek 2009) and it became accepted as a food source more recently as a result of food shortages associated with the Korean war of 1950-1953 (Podberscek 2009). While there has been international condemnation of the consumption of dog meat, in countries such as South Korea it remains popular (it is estimated that ~2.5 million dogs are slaughtered for food each year (Czajkowski 2014)) as a result of tradition (Kim 2008; Czajkowski 2014), and its purported effects on wellbeing and vigour

(Podberscek 2009). In Korea, 27% of the population, predominantly older males, eat dog meat (Kim 2008; Podberscek 2009).

In South Korea the demand for dog meat is serviced by over 750 000 dog meat farms (Czajkowski 2014), however as dogs are not legally classified as livestock (Livestock Products Sanitary Control Act (Republic of South Korea) 2010), the practice of dog farming is largely unregulated, and the welfare of dogs is unprotected (Czajkowski 2014). Reports suggest that the meat farm dogs are typically kept in cramped conditions, at high stocking densities, with inadequate nutrition and veterinary care (Kim 2007; Podberscek 2009). While there is no published evidence that documents the health and welfare of farmed dogs in any Asian country, studies relating to the protection of human health report parasite load and disease incidence in dog meat and carcasses (Kim *et al* 2009) that suggest that dog welfare may be compromised in such establishments (Mack & Fokidis 2017). However, quantitative evidence that dogs in meat farms are physiologically stressed is lacking.

In dogs, as in other animals, the physiological stress response to a demand or threat is mediated by increased activity within the sympatho-adreno-medullary (SAM) and hypothalamic-pituitary-adrenal (HPA) axes (Buhler *et al* 1978; Herman *et al* 2016). In dogs, the principle mediator of HPA axis activity is the hormone cortisol (Beerda *et al* 1996; 1997). While an acute cortisol response may be beneficial, cortisol is homeostatic, i.e. its acute actions serve to restore normal function, continued exposure results in systemic dysfunction including effects on cardiac output (Schubert *et al* 2009), blood pressure (Pickering 2007), metabolic demand (McCurley *et al* 2015) and systemic inflammation (LeMay *et al* 1990) that can ultimately increase the risk of morbidity and mortality (Öhlin *et al* 2004). As steroids are stably deposited into hair, as it grows, the quantification of hair cortisol concentrations provides a historical record of the physiological stress experienced by an animal during the period of hair growth

(Mastromonaco *et al* 2014). In dogs, this period equates to the 13-15 weeks prior to sample collection (Diaz *et al* 2006; Favarato & Conceição 2008; Welle & Wiener 2016). Hair cortisol has been shown to correlate with cortisol concentrations in blood, saliva, urine and faeces across a range of species, including: domesticated dogs and cats (Accorsi *et al* 2008); reindeer (Carlsson *et al* 2016); sheep (Stubsjøen *et al* 2015); primates (Davenport *et al* 2008) and humans (Wosu *et al* 2013; Papafotiou *et al* 2017). Furthermore, studies in a range of species have shown changes in hair cortisol concentrations in association with experimental stimulation of the HPA axis, living and social conditions, nutrition and disease (Heimbürge *et al* 2019). As such, hair cortisol may be a tool with which to assess chronic stress and/or the effects of poor welfare conditions.

The aim of this study was to assess if dogs surrendered from meat farms in and close to the city of Seoul South Korea exhibited higher concentrations of cortisol, in hair, relative to pet dogs in and close to Seoul. Higher concentrations of cortisol in hair samples collected from meat farm dogs would indicate chronic activation of the hypothalamic-pituitary-adrenal axis, indicative of chronic stress and/or poor welfare.

Materials and Methods

The study was approved by the University of Glasgow Animal Ethics Committee. Informed consent for the collection of hair samples was obtained from all owners of dogs included in the study.

Study animals

Pet dogs (n = 84), with no overt health problems were recruited to participate in this study by solicitation of the general public from pedestrianised streets in Seoul, South Korea. To be eligible to be included in the study the pet dogs had to have been resident within their current home for a minimum of five months to ensure a stable home environment (Radosevich *et al* 1989). As cortisol concentrations vary significantly between puppies and adult dogs (Palazzolo & Quadri 1987), dogs younger than five

months old, based on the absence of adult canine teeth (Kremenak 1969), were excluded from the study. Breeds represented in the pet dogs group included, beagle (n = 2), bichon frise (n = 3), border collie (n = 6), chihuahua (n = 2), cocker spaniel (n = 4), corgi (n = 1), crossbreed (n = 4), dachshund (n = 1), English setter (n = 1), golden retriever (n = 3), Japanese spitz (n = 7), Korean jindo (n = 1), Labrador retriever (n = 7), lhasa apso (n = 2), Alaskan malamute (n = 1), Maltese terrier (n = 6), papillon (n = 1), pomeranian (n = 9), poodle (n = 6), Pyrenean mountain dog (n = 1), Shetland sheepdog (n = 2), shiba inu (n = 10), shih tzu (n = 3) and Yorkshire terrier (n = 1).

Access to meat farm dogs (n = 86) was provided within one week of dogs coming into the ownership of two charities; Save Korean Dogs and Humane Society International (HSI). With regard to both charities, the dogs included in this study had been voluntarily surrendered to these organisations for rehoming and had no overt health problems. The dogs included represented 10 farms from the region in and close to the city of Seoul. For each farm, the number of dogs per farm, sex, breed, hair colour, average number of dogs per cage, and date of sample collection (summer/winter) was recorded (Table 1). The breeds represented in the meat farm dogs included Korean jindos (n = 61), cane corso (n = 2), corgi (n = 1), husky (n = 1), Japanese tosa (n = 1), Labrador retriever (n = 8), mastiff (n = 1), newfoundland (n = 1), poodle (n = 1), Pyrenean mountain dog (n = 8) and shiba inu (n = 1).

For both populations of dogs, hair samples (~50mg) were collected from the thigh, as described previously (Mesarcova *et al* 2017). The ischiatic region was chosen as it has consistent hair growth (Gunaratnam & Wilkinson 1983; Bennett & Hayssen 2010), and it is less traumatic for unsocialised dogs (Yelland & Whelan 2011). Hair was cut as close to the skin as possible with scissors, rather than being plucked or clipped, as inclusion of the hair follicle can distort cortisol concentrations (Gow *et al* 2010), and clipping can be a stressor for unsocialised dogs (Beerda *et al* 1997). Hair samples were stored at room temperature in individual labelled plastic zip-locked bags as described previously (Accorsi *et al* 2008) until assayed for cortisol. Cortisol analysis was blinded by allocation of random sample identity numbers to samples from both the meat farm and pet dogs, which were only decoded at the point of statistical analyses.

Hair Cortisol Analysis

Cortisol was extracted from hair samples using a modification of previously published methods (Accorsi *et al* 2008; Bennett & Hayssen 2010). Briefly, each sample was washed twice, for twenty-seconds, in 20ml of 100% ethanol to remove external sebum and sweat that could contain cortisol. Hair samples were finely cut with scissors (~2-5mm), frozen in liquid nitrogen and powdered using a Retsch Mixer mill (Retsch Mixer Mill MM400, Retsch, Haan, Germany). Approximately five milligrams of each powdered hair sample was transferred to a glass tube vial and 2ml of 100% HPLC grade methanol added. Vials were capped and placed into a heated orbital shaker (Stuart orbital incubator S150 (Cole-Parmer, Staffordshire, UK)) at 53°C, 200rpm, for 18 hours. Samples were then centrifuged, and a 375ul aliquot of the supernatant was removed, placed in a clean glass tube and dried down. Cortisol was assayed in duplicate using a commercially available ELISA (Cortisol express ELISA Kit, Cayman Chemicals, Ann Arbor USA), according to the manufacturer's instructions, following reconstitution of the dried sample in the assay buffer supplied in the kit.. Use of the ELISA was validated by demonstration of parallelism, using a 6 point serial dilution of a pooled sample of powdered hair from the pet and meat farm dogs, relative to the standard curve. Samples were assayed over six ELISA plates, and cortisol concentrations calculated using Assayzap software (Biosoft, Cambridge, UK). Assay sensitivity averaged 40.8 ± 3.3 pg/ml and inter- and intra-assay coefficients of variation averaged 5.47 and 6.51% respectively. Cortisol results are all expressed relative to the weight of hair extracted (pg cortisol/mg hair).

Statistical analyses

All statistical analysis was completed with R software (Version 3.2.5, © 2015 The R Foundation for Statistical Computing Platform) using the RStudio interface (Version 1.0.136, © 2009-2015 RStudio Inc.). Two models were created using the lme4 package (Bates 2010) with likelihood ratio tests to identify which interactions or variables were significant in the models. A backwards stepwise manual model building process was implemented for both models, where only those

variables that retained statistical significance (likelihood ratio tests) were considered for inclusion in the final models. Sex was included as an explanatory variable, as there are known sex differences in mammalian cortisol concentrations (Kenagy & Place 2000; Romero *et al* 2008; Bechshøft *et al* 2011). The hair life cycle in dogs can vary dependent on breed (Diaz *et al* 2004; Favarato & Conceição 2008; Welle & Wiener 2016) so the effects of breed on hair cortisol concentrations was also tested. Lastly, colour was considered as evidence exists in dogs that dark (eumelanin) hair sequesters less cortisol than light (pheomelanin) hair (Bennett & Hayssen 2010). An interaction between sex and group (pet or farmed dogs), breed and colour, breed and group, and group and colour were included; season was also considered as a random effect as hair growth and shedding rate can increase during summer months, in cross-bred dogs (Gunaratnam & Wilkinson 1983) and goats (*Capra aegagrus hircus*) (Ibraheem *et al* 1994), suggesting that cortisol concentrations in hair during the summer would depict a shorter timeline of chronic stress than in winter. In the first linear mixed effects model, the difference between groups (pet and meat farm dogs) were tested as the main fixed effect, and sex, breed, season and hair colour as random explanatory variables. In the second model, only the farmed dogs were included; this model tested the effects of breed, season, hair colour, farm identity, number of dogs/farm, and number of dogs/cage. These explanatory variables were included as individual farms can have different housing, husbandry methods and stocking densities that may impact dog stress (Sandri *et al* 2015). Furthermore, the number of dogs/cage and dogs/farm could influence the level of care each individual may receive (Fraser 2005), and dogs in solitary housing (Protopopova 2016; Grigg *et al* 2017) and other farmed mammals kept in high stock densities (Knowles *et al* 1988; Tarrant *et al* 1992) can be prone to increased stress. The statistical significance of each variable and pairwise interaction was tested using ANOVA. Two-sample t-tests were used to test the significance of individual factors. Within the hair

cortisol data from the Korean jindos, a 2 way ANOVA was used to look for effects of hair colour. All data are represented as mean \pm SEM. Statistical significance was defined as $P < 0.05$.

Results

The mean hair cortisol concentrations measured in the dogs rescued from meat farms (40.81 ± 4.58 ng/mg hair) was significantly ($P < 0.001$, $t = -4.63$, $df = 99.62$) higher than that measured from samples collected from South Korean pet dogs (18.83 ± 1.35 ng/mg). Sex, breed and hair colour were without significant effect on mean hair cortisol concentrations. Within the farmed dogs, there were no statistically significant effects of farm identity, number of dogs/farm or dogs/cage on mean hair cortisol concentrations. The breed most represented in the meat farm dogs was Korean jindo. Korean jindo has 4 main colour variants, black, white, agouti and brown. Comparison of hair cortisol concentrations within the four colour variants indicated that hair cortisol was significantly higher in white compared to black dogs ($P < 0.010$, $t = 2.83$, $df = 25.53$), however, all other comparisons of colour variations (black = 21.54 ± 5.78 pg/mg ($n = 5$), white = 49.93 ± 8.18 pg/mg ($n = 42$), agouti = 54.09 ± 23.95 pg/mg ($n = 5$), brown = 44.30 ± 13.40 pg/mg ($n = 10$) were not significantly different.

Discussion

This research is the first to report hair cortisol concentrations, as a marker of stress, and thus, welfare status, of farmed and pet dogs in South Korea. The results demonstrate that dogs surrendered from meat farms had evidence of higher levels of HPA activity and therefore higher levels of chronic physiological stress during the 3 to 4 months preceding sample collection, compared to South Korean pet dogs. The higher hair cortisol concentration observed in meat farm dogs was independent of the farm from which the dogs came, or the size and stocking density at the farm; the breed, colour and sex of the dog. The observed effect, therefore appears to be highly consistent across a diverse population of subjects. Previous work

in dogs has shown that a variety of environmental conditions can result in elevated cortisol secretion, including spatial and social restriction (Beerda *et al* 1996; Grigg *et al* 2017), conspecific aggression (Kelly & Vitousek 2017), lack of stimulation (Schipper *et al* 2008; Kiddie & Collins 2015), starvation (Fujiwara *et al* 1996), extreme temperatures (Hume & Egdahl 1959; Assia *et al* 1989) and dehydration (Slater *et al* 1963). While the detailed conditions in which the farmed dogs were kept were not documented for the dogs included in this study, the farmed dogs are more likely to have been exposed to these known stressors than the sampled pet dogs. For example, farmed dogs in South Korea are normally kept outside with little shade and are therefore subject to harsh environmental temperatures, whereas pet dogs are more likely to reside in buildings that provide shade, heating and air conditioning, where appropriate.

The results of this study indicate a robust elevation in hair cortisol, in meat farm relative to pet dogs, as the mean hair cortisol concentrations seen in the meat farm dogs indicate that the dogs had been exposed to either very high acute stress or chronically elevated stress levels during the 3-4 month period prior to sample collection. Meat farm dogs will normally spend their whole lives on one farm and their transportation away from their farm and introduction to a new environment could be argued to be an acute highly stressful event that would result in high levels of HPA activity, as evidenced in laboratory dogs (Kuhn *et al* 1991; Beerda *et al* 1997). However, this cannot explain the findings observed in this study. The meat farm dogs were voluntarily surrendered to the rescue charities as such the hair samples were collected at the charity centres, within the first week after being surrendered. In each case the hair was cut with scissors so represented the hair grown while the dogs were present within the meat farms as opposed to the 1-3 mm of hair closest to the root which could reflect the stress associated with changes in their environment.

Across the samples collected for this study, sex and breed had no effect on hair cortisol concentrations. The absence of a sex difference in hair cortisol concurs with research into the effects of starvation on cortisol in dogs (Reimers *et al* 1990). While there have been few, if any, systematic studies of breed differences in circulating cortisol concentrations, it has been suggested that some breeds of dog are more sensitive to stressors than others (Mahut 1958; Storengen & Lingaas 2015) and that smaller dogs have higher circulating cortisol than larger dogs (Reimers *et al* 1990). In this study, we found no effect of breed, but it should be noted that many breeds were only represented by one or a very small number of dogs in our sample. More importantly, the pet dog group, which was biased towards smaller dogs, had cortisol concentrations that were lower than the meat farm dogs. It has also been reported that hair cortisol concentrations can vary with coat colour (Mesarcova *et al* 2017). We therefore tested for such an effect within our data, specifically within the Korean jindos which have distinct coat colour polymorphisms. While we did not see an overall effect, we found that hair cortisol was significantly ($P < 0.01$) higher in white (22.61pg/mg $n = 42$) compared to black (17.98pg/mg $n = 5$) hair. This result is in agreement with previous reports in dogs (Bennett A & Hayssen 2010) cattle (Burnett *et al* 2014; González-de-la-Vara *et al* 2011) and chimpanzees (Yamanashi *et al* 2013). However, other studies in cattle have reported either no effects of colour on hair cortisol (Nejad *et al* 2017) or contrary findings (Tallo-Parra *et al* 2017) and as such further investigations are required.

Conclusions and animal welfare implications

The results demonstrate that dogs from meat farms have higher hair cortisol concentrations, indicative of chronic cumulative stress, relative to South Korean pet dogs. While this is only one measure of the physiological stress response in these animals, chronically elevated cortisol is associated with adverse health outcomes and increased risk of morbidity and mortality (Öhlin

et al 2004). Overall, the results of this study provide the first quantitative evidence that dogs in meat farms in South Korea are kept in conditions in which their welfare is likely to be compromised. The results, therefore, support the introduction of changes in the regulations, policy and legislation surrounding the farming of dogs for meat. Our findings could also inform those who consume dog meat, of the potential long-term physiological suffering of these dogs, and could bring about a reevaluation of the cultural beliefs regarding the consumption of dog meat.

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425 Table 1. Details of the numbers, sex, breed, density of dogs and date of sample collection (summer/winter), in each of the 10 farms from which meat
 426 dogs were surrendered and a breakdown of the colour variants for the Korean jindos.

427

Farm ID	Total number	Number of females (F)	Number of males (M)	Cage density on farm	Season	Number of Korean jindos	Number of Korean jindos of each hair colour variants (sex)			
							White	Brown	Black	Agouti
a	4	1	3	1	summer	4	0	2 (F=1, M=1)	0	2 (M)
b	3	3	0	4	winter	3	2 (F)	1 (F)	0	0
c	3	2	1	5	summer	3	2 (F)	1 (M)	0	0
d	18	9	9	5	summer	13	9 (F=5, M=4)	2 (F)	1 (F)	1 (M)
e	47	22	25	3	winter	27	17 (F=12 M=5)	3 (M)	4 (F=1, M=3)	3 (M)
f	2	1	1	1	winter	2	1 (M)	1 (F)	0	0
g	1	1	0	3	winter	1	1 (F)	0	0	0
h	1	1	0	1	summer	1	1 (F)	0	0	0
i	5	3	2	5	summer	5	5 (F=3, M=2)	0	0	0
j	2	2	0	1	winter	2	2 (F)	0	0	0
totals	86	45	41	29		61	40	10	5	6

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